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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 16/00, G01N 33/53, A61K 39/385, 39/39, 49/00 — A1

(11) International Publication Number: WO 97/35884

(43) International Publication Date: 2 October 1997 (02.10.97)

(21) International Application Number: PCT/GB97/00855

(22) International Filing Date: 26 March 1997 (26.03.97)

(30) Priority Data:
9606425.8 27 March 1996 (27.03.96) GB

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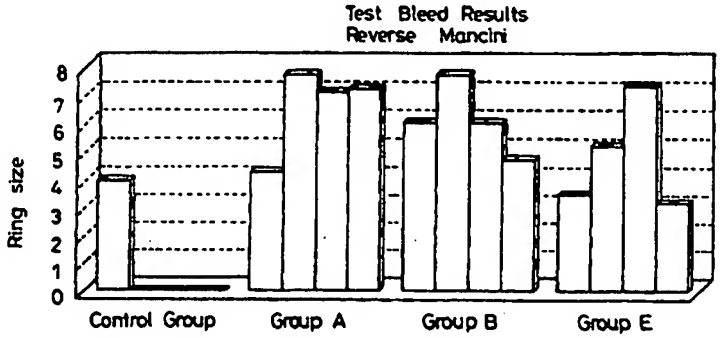
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IMPROVED PRODUCTION OF ANTIBODIES THROUGH THE USE OF ANTIGEN ANTIBODY COMPLEXES



	Sheep 1	Sheep 2	Sheep 3	Sheep 4
Control Group	4			
Group A	4.3	7.7	7.1	7.2
Group B	6	7.7	6	4.7
Group E	3.5	5.2	7.3	3.2

(57) Abstract

We have been able to make high affinity and high titre antibodies by administering not only antigen to an animal, but also a relatively low dose of antibodies to that antigen. These high affinity antibodies can then be used to make better immunological diagnostic test kits, or in the preparation of therapeutic substances, or in the treatment of patients. High affinity antibodies are administered to an antibody source in order to make even higher affinity antibodies.

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IMPROVED PRODUCTION OF ANTIBODIES THROUGH THE USE OF ANTIGEN ANTIBODY COMPLEXES

This invention relates to the production of antibodies that have high affinity and/or high titre, to test kits incorporating them, and to medical uses involving administering high affinity antibodies.

Prior Art Ways of Producing Antibodies

Antibodies for test kits, and other uses (e.g. therapeutic uses), can be produced by injecting an animal with the antigen in question, allowing its immunological response to produce antibodies, and then extracting the antibodies (antiserum) and incorporating them in a kit.

Monoclonal antibodies can be produced by stimulating in vivo and cloning appropriate B cells in vitro.

The titre of specific antibodies in an antiserum from the antibody source is important. If a high titre of antibodies specific to one antigen is produced in the antiserum (e.g. in sheep's blood) then there is a relatively lower proportion of other, unwanted, non-specific antibodies present in the antiserum sample (or other, non-wanted, antibodies will be at relatively lower concentrations). This means that, for example, an IgG extract from the antiserum has a reduced potential for non-specific interference when it is used to produce a test reagent.

It is common using known antibody production techniques to produce antisera of low specific titre that require extensive purification, for example by absorption and affinity purification techniques.

A further advantage of a high titre antiserum is that because the concentration of antibodies of the specificity that are wanted is high the cost of the production of antibody test reagents or antiserum is reduced and the speed of the purification process may be increased in some situations. It is conventional to inoculate a number of animals to produce antibodies and select the animal or animals that has or have the highest titre, or affinity, to extract large quantities of antisera from.

Antisera with high affinity are also desirable. A high affinity antibody can be used to increase the sensitivity of an immunological assay kit. A higher affinity antibody to an antigen, antigen X, can detect the presence of antigen X in a test sample at lower concentrations of antigen X than antibodies to antigen X that have lower affinity.

The production of antibodies with higher affinities than can be produced at present would, if possible, open up the possibility of testing for entire ranges of analytes that at present simply cannot be tested for using immunological test kits. Higher affinity antibodies would open up the possibility of testing for more analytes, and therefore being able to diagnose more disorders, or to detect anomalies at lower concentrations and therefore diagnose earlier, or to be able to use simpler test systems because the antibodies are of higher affinity.

Background to Medical Uses Involving Antibodies

High affinity antibodies have uses in passive vaccines (a high affinity antibody in a vaccine will work better than a low affinity vaccine), in diagnosing tumours and other problems (by localising a detectable substance at the selected region), in treating tumours and other problems (by localising therapeutic substances at the selected regions); and in combating unwanted effects from foreign chemicals (e.g. drug overdoses or snake venom).

tumours and other problems (by localising a detectable substance at the selected region), in treating tumours and other problems (by localising therapeutic substances at the selected regions); and in combating unwanted effects from foreign chemicals (e.g. drug overdoses or snake venom).

Some medicaments (e.g. vaccines) rely upon the immune system of the person (or animal) to make them work. For example an injection to protect against Hepatitis A involves introducing antigen from the Hepatitis A virus into the body so that the immune system will create antibodies to it and have memory B cells for two or three years ready to produce further antibodies to the "recognised" antigen of Hepatitis A (or at least a part of its protein structure) at short notice.

To generate good immunological resistance to a virus it may be necessary to have "booster" injections of the antigen some time after the first primary inoculation. For example it may be necessary to go back to the doctor a month after the first injection to get a second, follow up, injection and perhaps even a third injection later still. This is because the second injection needs to happen after the immune system has had time to complete its primary immune response so that it can exhibit a secondary response, at higher antibody titre and affinity levels, when the second injection occurs. Similarly the third injection, even later, is to elicit a tertiary response. This means that the memory B cells which persist for years will produce good high titre, high affinity, antibodies if called upon.

The invention in Broad Terms

We have discovered that if we administer an antigen to an antibody source and also administer, at the right dosage, antibodies to that antigen we

i. the realisation that if an antibody source is both immunised with an antigen and also has administered to it antibodies to that antigen we can make it produce higher affinity antibodies, and at a higher titre, than if it is simply immunised in the conventional way, thus allowing us to produce better antibodies;

ii. that once we have higher affinity antibodies we can make better diagnostic tests, and test kits; and

iii. that once we have higher affinity antibodies we can make more effective medical treatments that use antibodies, and can make more effective preparations for use in such treatments.

Producing High Affinity/High Titre Antibodies

According to a first aspect of the invention we provide a method of increasing the titre and/or affinity of an antibody sample extracted from an antibody source, the method comprising administering to the immunological system of the source both antigen to produce the desired antibodies and also antibodies to the antigen.

This has been found to increase the titre of antibodies to the selected antigen. It has also been found to produce antibodies of higher affinity. This technique can achieve both high titre and high affinity.

We administer about 0.1 to 500μg of antibody to a sheep along with about 50μg of antigen to produce good antibodies. We may administer up to 15 mg of antibody. We believe that administering higher doses of antibody achieves a higher affinity and titre, but the effect is not linear: there is diminishing additional benefit at high doses. In another embodiment we may

administer about 0.1μg, to about 1.0μg of antibody to a sheep (along with about 1μg of antigen) to produce antibodies. In a further embodiment we may administer from 0.01 to 25 μg, but prefer to remain in the range 0.1 to 10μg of antibody.

Possible theories to explain the Production of Good Antibodies

We have postulated possible mechanisms which could explain the beneficial result achieved by administering antibodies and antigen to the antibodies we want to produce. One theory is that affinity maturation is stimulated. B cells produce antibodies. The affinity of the antibodies produced by B cells in an immune response increases with time by a process of competition and survival of the highest affinity clones.

B cells need to bind to a follicular dendritic cell (FDC) in association with antigen in order to divide and reproduce. They have a relatively short time to do this, perhaps 4 hours or so, before they die. In order to bind to a FDC the (activated) B cell needs to compete with other B cells.

Thus those B cell mutations which produce the high affinity antibodies remain at the FDC reproduction centres for longer, and so they increase in population. This produces a drive for the production of higher affinity antibodies.

By injecting high affinity antibodies into the animal we ensure that there is a population of high affinity antibodies in the immune system, and so the immune system cannot, right from the start, produce low affinity antibodies to the antigen because the injected high affinity antibodies that are introduced compete for space at the FDC and win the competition. This effectively

prevents the immune system from producing low affinity antibodies which are associated with the traditional primary antibody response.

The measured titre of antisera produced using the present invention may be higher because the measured titre includes less antibodies which have a low affinity. The immune system is producing a higher percentage of high affinity (titre-contributing) antibodies and so its perceived titre is higher than would conventionally be the case. This competition by activated B cells is known as affinity maturation and is known as a phenomenon in itself, but its application in the production of high affinity antibodies by administering antibodies to the antigen in question to the source has not been appreciated before. The circulating antibodies may drive the affinity maturation.

A second theory is that we could be switching off B cells by inoculating with antibodies to the antigen, but expanding the number of primed T cells. The injected antibodies may bind to B cells, switching them off, while still stimulating T cells. When a subsequently injected antigen does activate a naive B cell there is a super abundance of T cells to promote antibody production. The antigen will again bind to the "best" (high affinity) B cells.

A third theory is that we could conceivably be achieving an increase in macrophage antigen uptake. This increases T cell stimulation, which in turn increases the production of antibodies to the antigen by B cells.

Thus one possible way of looking at the invention is as a way of increasing macrophage antigen uptake by administering to the antibody source (e.g. host animal) antibodies to the antigen, as well as the antigen (i.e. immune complexes).

Other preferred steps taken to produce good Antibodies

Preferably the antibody source is an animal, preferably a mammal, such as a sheep. The animal may be a human, especially when the antibodies produced are going to be used in therapy or in vivo diagnosis.

The administered antibodies are preferably administered intravenously to the animal antibody source, and the antigen is preferably administered subcutaneously to the animal antibody source.

The antibody source may be a cell culture. We may consider producing monoclonal antibodies, but prefer to produce polyclonal antibodies.

We may administer antigen on day zero and no antibody then. We may administer antibodies to the antigen that has been administered a short time after the administration of the antigen. A short time may be a matter of days (e.g. 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, or a few more). We have had good results administering the antibodies 8 days after administering the antigen.

We may administer both the antigen and antibodies on day zero. They may be administered simultaneously, or substantially simultaneously. We may even administer the antibodies a short time before the antigen.

We may prefer to administer antibody to the sheep in staged administrations separated by time. We may increase the affinity or amount (or both) of a second, or subsequent, administration of antibody to a source of antibodies (e.g. sheep or humans). In this way we can administer a low dose of antibodies, e.g. 0.1-1μg, (and the antigen) initially within a short time of each other to start the production of good antibodies and then, some time (e.g. days, possibly 1,2,3,4,5,6,7,8,9,10 or more days) later administer subsequent

doses of antibodies, with the suceeding doses having more or higher affinity (or both) antibodies. These subsequent doses of antibodies may stimulate the production of even higher affinity antibodies in the source (e.g. sheep), and do not shut down/inhibit the production of antibodies as they might do if they were given initially (too high a dose of antibodies to the antigen administered initially can inhibit antibody production, rather than stimulate it).

The antibodies administered to the source at least in the first administration are preferably administered to be at a low level in the source so as not to switch off B-cell production for these antibodies.

The antibody source may be tolerised to another, or a plurality of other, antigens (typically by administering the other antigens to the source, possibly repeatedly to tolerise).

The antibody source may have administered to it another, or a plurality of other, antibodies to antigens other than the antigen to which the desired antibodies being produced respond. The antibodies administered to the source may have a high affinity.

When we boost an antibody source (e.g. sheep) with antigen some time after day zero we may prefer to do it about three to six weeks afterwards, or at least two weeks afterwards.

We may harvest the antibodies produced by the source about a week after the last boost of antigen.

The antigen administered may be carried by a conjugate. If there is a second administration of antigen the second administration may have the antigen presented in a different form from the first administration (e.g. with a

conjugate, or with a different conjugate if the first administration had a conjugate).

It may be desirable to ensure that the source has not previously encountered the selected antigen so as to ensure naive B cells.

Other Definitions of Invention Relating to Producing Antibodies

According to another aspect the invention resides in the use of a preparation including an antibody to an antigen in the production from an antibody source of an extract/sample having high/increased affinity antibodies.

According to a further aspect the invention resides in the use of X to do Y, where

X is antibody and antigen, and

Y is a) increase titre

or b) increase affinity

Preferably the antibodies administered to the source are of high affinity. Preferably above a threshold affinity of 10^{-9} litres/mole (L/mol), and perhaps at about 10^{-10} L/mol, or above.

High affinity antibodies are administered to the source in order to make more antibodies of even higher affinity (or at least more antibodies of substantially the same or higher affinity).

We suggest that giving low concentrations of an antibody to an antigen at the time of immunisation with the antigen (or within a short while of each other) gives better affinity, and also higher titre.

We have discovered that inoculating an antibody source (e.g. host animal) with antibodies that it is intended to produce, and inoculating with the antigen, gives a higher titre, and in the tests we have conducted, a higher affinity. We have conducted tests on IgG1 antigen in large animals, such as sheep, for the production of polyclonal antisera. The tests support the invention. [This does not exclude the use of this technique for any antibodies including production of monoclonal antibodies.] It will be appreciated that these test results show that the blood of the sheep has good antibodies in it. Whether these are extracted and used to produce a medicament/vaccine, or left in the sheep to fight infection, the presence of good, improved, antibodies in the sheep is nevertheless demonstrated by the test results.

Another aspect of the invention resides in the use of a higher than normally found in the human body concentration of high affinity antibodies (or of antibodies that are purer than are normally found in the human body) to an antigen in the preparation of a diagnostic reagent, or therapeutic substance, for use in the identification of a disorder associated with the antigen in a sample from a test subject, or for use in the treatment of a disorder associated with the antigen.

We also seek protection for a package of antibodies, possibly in a pharmaceutically acceptable carrier; a diagnostic method; and kit; involving high affinity antibodies produced using the first aspect of the invention.

Medical Uses of High Affinity Antibodies

According to a another aspect of the invention we provide a vaccine, or other medicament containing an antigen, comprising an antigen A, and antibodies to antigen A, the dose of the antibodies being such that in use they stimulate the immune system of the vaccinated subject to produce higher

affinity antibodies to antigen A than they would if just antigen A were injected into the subject.

Preferably the amount of the antibodies is 0.001μg to 15mg, and may possibly be in the range 0.01 to 10μg.

The antigen A and the antibodies to it are preferably in separate pharmacologically acceptable carriers, which may be adapted to mix during the injection process.

Alternatively the antigen A and the antibodies to it may be present in the same pharmacologically acceptable carrier.

The vaccine is preferably labelled as having an antibody in it.

The vaccine is preferably provided in a predetermined dose in a cartridge or vial for a syringe or injector, or is pre-provided in a syringe or injector.

Preferably the antibodies have an affinity of 10^{-9} L/mol or above.

Preferably the antibodies have an affinity of 10^{-10} L/mol or above.

Preferably the antibodies have an affinity of 10^{-11} L/mol or above.

The vaccine is preferably adapted to treat disease X, and the antigen A is at least a part, and perhaps substantially all, of the protein structure of the agents that cause disease X;
where X is from the list:-

Rubella

Rabies

Diphtheria

Tetanus

Whooping Cough

Typhoid

Hepatitis A

Hepatitis B

Hepatitis C

Tuberculosis

Chicken Pox

Mumps

Scarlet Fever

Yellow Fever

Aids

CJD

Polio

Cholera

The vaccine may also include an adjuvant.

The vaccine may be part of a kit further including one or more of: an injector; or instructions on how to use the vaccine; or a plurality of containers containing a predetermined amount of vaccine adapted to treat a plurality of patients.

According to another aspect the invention provides an immunological test kit adapted to diagnose a disorder that is indicated in a sample taken from a patient by the presence of, absence of, or variation in the amount of an antigen,

the kit comprising antibodies to said antigen that have been made in accordance with the present invention.

The kit preferably has instructions on how to use it.

The kit preferably has control material and/or reference standard material provided in it.

The present invention further provides a preparation for use in a test to identify the presence of an antigen, the preparation having antibodies of high affinity made in accordance with the present invention.

The antibodies are preferably present at a concentration of 0.1 to 50 mg per millilitre, and most preferably at about 1 to 5 mg per ml.

The preparation may be labelled with its purpose.

The present invention provides the use of a preparation including an antibody to an antigen in the production from a source of an extract/sample having high/increased affinity antibodies.

The present invention provides the use of an antibody (antibody 1) to an antigen (antigen 1) in an administration to an animal or culture, along with antigen 1, in the preparation of a sample extracted from the animal or cell culture, and subsequent use of the sample in the preparation of a diagnostic reagent for use in the identification of the presence of the antigen 1 in a test sample from a test subject.

The present invention further provides the use of an antibody (antibody 1) to an antigen (antigen 1) in an administration to an animal or cell

culture, along with antigen 1, in the preparation of a sample extracted from the animal or cell culture and, the subsequent use of said sample in the preparation of a medicament for use in the treatment of a disease, the disease preferably having an antigen, antigen 1, associated with it.

According to another aspect the present invention provides a method of vaccinating or inoculating an animal (e.g. human) against a disease D, the method comprising vaccinating with a vaccine against disease D having antigen of disease D, or closely associated with an antigen of disease D, and also introducing to the animal antibodies to the antigen vaccinated.

Preferably the antibodies are introduced with a dosage of 0.001μg to 15mg, preferably 0.01 to 1 mg, and in one embodiment 0.01 to 10μg.

The antibodies administered preferably have an affinity of at least 10^{-9} L/mol, and most preferably of 10^{-10} L/mol or above.

The present invention also provides a method of improving the performance of a vaccination or inoculation, the method comprising giving a vaccination of vaccine material that hitherto did not work as well as it might have and also administering antibodies to at least some of the vaccine material, thereby generating higher affinity antibodies in the vaccinated subject.

The present invention further provides the use of both antigen A and antibodies to antigen A in the preparation of a medicament or vaccine for the treatment (prophylactic or therapeutic) of disease X, where the agent that causes disease X has antigen A (or an antigen very similar) in its protein structure.

The disease X may not be a disease in its normal sense but is instead a disorder associated with the presence of antigen A (e.g. where antigen A is a toxin for example a snake venom, or the results of a drug overdose).

The present invention provides in a further aspect a series of planned injections at spaced apart intervals to vaccinate against a disease X that has associated with it an agent having a structure of antigen A, comprising the administration to a subject being vaccinated of both the antigen A and antibodies to the antigen A in order to improve the immune response of the subject to the first injection (or early injections in the planned series) thereby avoiding the need for at least one of the later traditionally performed injections.

The use of the administration of both antigen A and antibodies to antigen A may reduce the number of injections required from 3 to 2.

The use of the administration of both antigen A and antibodies to antigen A may:-

a) reduce the number of planned injections to 1; or

b) reduce the number of planned injections by 1 (or more). (for example from 4 to 3, 3 to 2, or 2 to 1).

Another aspect of the invention provides a method of improving an inoculate comprising ensuring that the inoculate has both an antigen and antibodies to the antigen.

The invention further provides a method of diagnosing localised disorders (e.g. tumours) comprising introducing into the patient antibodies that are specific to one or more epitopes of the disorder (e.g. tumour) and having

the antibodies associated with a readily detectable agent so that when a diagnostic scan is performed the readily detectable agent is localised at the site of the disorder to make it detectable, and wherein the efficiency of the method is increased by using antibodies that are of high affinity.

The antibodies may be of a higher affinity than can be obtained simply by introducing into a naive antibody source antigen (of the desired epitopes) and extracting the antibodies so generated.

The antibodies preferably have an affinity that is 10^{-9} L/mol or above.

The invention further provides the use of high affinity antibodies in the preparation of a diagnostic substance for the diagnosis of (or improved imaging of) a localised disorder (e.g. a tumour), the antibodies having been made in accordance with the present invention.

According to a further aspect the invention provides a method of targeting the delivery of a therapeutic substance to some cells but not other cells in vivo, the method comprising using antibodies specific to one or more epitopes of said some cells, (which epitopes are not present on said other cells), and associating with the antibodies the therapeutic substance, the method further comprising using high affinity antibodies made in accordance with the present invention.

The antibodies are preferably of higher affinity than can usually be obtained by administering the epitopes to a naive antibody source and extracting the antibodies to the epitopes from the source.

The antibodies preferably have an affinity of at least 10^{-9} L/mol.

The invention provides the use of high affinity antibodies in the preparation of a medicament for the treatment of a disease or disorder, the high affinity antibodies concentrating the medicament in specific regions better than with conventional lower affinity antibodies, and the antibodies being made in accordance with the present invention.

The invention further provides a method of diagnosing a disorder in which a protein is produced, or is absent or diminished, comprising taking a test sample from a subject animal and using an antibody to the protein to identify its presence, and in which the antibody has been produced in accordance with any preceding invention.

The invention further provides a medicament or therapeutic substance for use in the treatment of disorder associated with an antigen, antigen A, the medicament having antibodies to antigen A, said antibodies being of high affinity and being produced in accordance with the present invention and being present at a level such as to have a beneficial result when the medicament is administered to a patient.

Preferably there is also a physiologically active substance.

The physiologically active substance may be associated with the antibodies (e.g. conjugated with them).

The medicament is preferably substantially free of other physiologically active substances (beyond said antibodies and if present said [selected] physiologically active substance).

Preferably the antibodies that are used, along with the antigen, are high affinity antibodies. Preferably they have an affinity of at least 10^{-9}, or 10^{-10}, or even 10^{-11} L/mol (although these are currently difficult to make).

The affinity of an antibody may also depend upon the way in which the antigen is introduced to be the source of antibodies (e.g. intravenously or subcutaneously), and the epitopes on the antigen molecule (repeating epitopes produce high affinity). High affinity antibodies, made using the present invention, have significantly higher affinity than antibodies made by a comparable source by introducing just antigen in the traditional way.

The antibodies are preferably given to the subject at a dose of 0.001μg to 15 mg (for a human adult). The amount given of high affinity antibody is chosen to be such as to cause the immune system of the recipient to produce good high affinity antibodies (or even higher affinity antibodies than are introduced to the subject).

According to another aspect of the present invention we provide a use of the technique of producing high affinity antibodies for reducing the number of immunisations necessary in a vaccination regime to build up the immune response to protect the patient.

This decrease in the number of immunisations required reduces vaccination costs as well as being more desirable for the patient as fewer trips to the doctor and fewer injections are needed. This technique could increase the effectiveness of immunisation.

For use in medicaments the antibody source and patient must be of the same species. Alternatively the antibodies can be humanised.

It will be appreciated that introducing antibodies to the patient with a view to the added antibodies themselves having a direct beneficial effect is a slightly different concept to that of introducing antibodies so as to encourage the immune system to generate more of its own, good, antibodies.

The dosage of antibodies for direct therapeutic or diagnostic effect are not restricted by the desire to avoid switching off the production of antibodies by the immune system and can be larger.

A higher affinity antibody may have an association/disassociation constant such that we could expect the time for 50% of antibodies to be disassociated to be of the order of 10 or 20 hours, instead of what is possible now (3 or 4 hours). This makes treating tumours, or cancers, by using toxic (chemically toxic or radioactively toxic) antibodies more practical. It may make it possible to get a lethal dose (or LD50) dose to tumours without approaching anything like the same dose elsewhere in the body.

We could also use antibody and radiation - activated cell killing agents. The radiation could be light (optical, infra red, or ultra violet), or microwave, x-ray, or particle radiation.

Examples showing Specific Embodiments of the Invention

The invention will now be described by way of example only with reference to the experiments described below and to the accompanying Figures, of which:-

Figures 1 to 3 show test results of a first set of tests on sheep;

Figures 4 to 7 show test results of a second set of tests on sheep;

Figure 8 shows a two vial vaccination combination;

Figure 9 shows an injector;

Figure 10 shows an injector and two cartridge combination; and

Figure 11 shows a kit having a plurality of predetermined vaccine doses.

First Set of Tests

Six groups of five sheep were immunised with human IgG, at the same time each group was administered intravenously with varying doses of affinity purified sheep anti-Human IgG. Doses given were as follows:-

Group A	25 μg
Group B	1 μg
Group C	0.1 μg
Group D	0.01 μg
Group E	0.001μg
Group F	0

Six weeks later the immunisation and administration of i.v. anti-Human IgG was repeated using the same doses as quoted above.

One week after the second immunisation a 10mL sample of blood was taken from the sheep, after extraction of the serum, the following tests were performed on the antiserum samples.

1. Relative titre was determined by Reverse Mancini.

2. Performance on Hitachi analyser assessed.

3. Performance on BNA analyser assessed.

Results were as follows:-

Figure 1:- Relating to Table of all results.

Row 1	Sheep Number
Row 2	Treatment group
Row 3	Dose of anti-Human IgG (μg)
Row 4	Top reading on Hitachi
Row 5	Bottom reading on Hitachi
Row 6	Top reading on BNA
Row 7	Bottom reading on BNA
Row 8	5µl Reverse Mancini diameter (mm)
Row 9	1µL Reverse Mancini diameter (mm)

Figure 2

Graph of BNA bottom point versus antibody dose.

Figure 3

Graph of relative titre versus antibody dose. Relative titre is expressed as the square of the 5µl Reverse Mancini ring diameter.

The bottom reading on the BNA is a good measure of affinity. As can be seen in Figure 2 there is a significant antibody dose effect, administration of antibody increases the affinity of the response, with the 1µg dose being optimal in this experiment. From Figure 3 it can be seen there is also a relationship between relative titre as assessed by the square of the 5µl Reverse Mancini ring diameter and the antibody dose. In this case 0.1µg would appear to be the optimal antibody dose. Administration of i.v. antibody to the antigen at the time of immunisation gives an increase in the titre and affinity of antibody response of the host animal.

Second Set of Tests

Four groups of four sheep were immunised with human IgG. Eight days later each sheep was administered intravenously with varying doses of affinity purified sheep anti-human IgG. Sometime afterwards a booster dose of the human IgG was administered to each sheep.

A control group was immunised with human IgG and sometime later received a booster of human IgG. No antibody was administered to the control group sheep.

One week after the booster dose of human IgG a 10mL sample of blood was taken from each sheep, after extraction of the serum the following tests were performed on the anti-serum samples.

1) Relative titre was determined by Reverse Mancini
2) Performance on a Hitachi analyser was assessed.

Results were as follows:-

Figure 4 - Details of doses and times of antigen and antibody administration

Figure 5 - Relating to the table of all results.

Column 1	Sheep number
Column 2	Treatment group
Column 3	1μL and 5μL Reverse Mancini diameter (mm)
Column 4	Hitachi analyser readings

Figure 6 - Graph of relative titre versus antibody dose. Relative titre is expressed as the square of the 5μL Reverse Mancini ring diameter.

Figure 7 - Graph of bottom Hitachi calibration point versus antibody dose.

Reverse Mancini is a gel method which gives a relative measure of antibody titre and the bottom calibration points on the Hitachi analyser provide a measure of antibody affinity.

Figure 7 shows that the affinity of the antibody produced is increased by the intravenous administration of anti-Human IgG subsequent to immunisation. A comparison of group A with the control, which have identical conditions with the exception of antibody dosage, shows that the administration of antibodies produces antibodies of a higher affinity. This increased affinity in

the antibodies is also seen in group B, however it appears that increasing the dose of antibodies slightly, as in group B compared with group A, has little effect on the affinity of the antibodies produced.

The affinity of the antibodies produced by group E is generally much lower than these of group B, which has identical conditions with the exception of the antibody dose, and we believe that this is due to the huge dose of antibodies administered to group E after immunisation inhibiting the production of high affinity antibodies.

Figure 6 shows that the titre of the antibodies produced is also increased by the intravenous administration of antibodies, this is best seen by comparing groups B and E. It can also be seen that administering a huge dose of antibodies after immunisation does not have as marked an affect on titre as it does on affinity. The titre of group E is only slightly lower than that of group B.

For the avoidance of doubt, we prefer to take an animal (e.g. an adult sheep), introduce antibodies to an antigen, and the antigen at or about the same time (within one to ten days of each other). This can be repeated as necessary (or possibly just antigen or antibodies administered later). We then take blood from the animal some time later (e.g. a week) and extract from that blood an antibody extract containing antibodies produced by the animal. We then propose to purify the extract, for example using an affinity purification technique or IgG fractionation, and put the purified extract into vials, labelled with their contents, and put the vials in immunological test kits. The animal is left to recover, and able to donate more blood in the future.

An alternative use of the antibodies once we have produced them is in the preparation of a medicament for the treatment of an ailment (that the

antibodies alleviate). The use of such antibodies in such a preparation constitutes another aspect of the invention. We may provide an inoculate that contains antibodies made in accordance with the first aspect of the invention.

It will be appreciated that when the invention is applied to diagnostic methods it may not be applied to the human or animal body as such, but rather in vitro, to a sample (e.g. blood and urine) extracted from the body.

In the above example and all other trials this technique has been used in the production of antisera in non-human hosts, however, another potential use of this technique is in vaccination of humans. For many/most vaccinations a course of three or more immunisations is necessary to build up the immune response which protects the patient. Use of this technique could reduce the number of immunisations necessary reducing vaccination costs dramatically, and further more could increase the effectiveness of immunisation.

In many third world countries programmes of mass vaccination are not as successful as they might be because when the vaccination regime requires a plurality of injections it is difficult for the subject to ensure that they receive subsequent injections of the intended course. It is not unknown for the subject to receive only the first injection of a multi-injection course. This is understandable where the subject might have to walk for a day or so to the hospital (or far further), and might not have a postal system to remind them to attend the clinic. It is by no means unknown for patient in the developed world to forget to come for subsequent injections in a programme, or find it too much trouble to come. A lot of time of medical staff, and expense, is taken up giving later injections of a planned course.

If our present invention can eliminate the need for even one injection from a course, by making the first injection (and perhaps later injections if they are given) more effective, this will be of great benefit.

Furthermore, there are some diseases where it is theoretically believed vaccination will reduce the number of people who contract the disease, or help to cure those who have it, but where in practice the vaccines that are currently available are less than 100% satisfactory. Some vaccines work a little, but not very well. Other vaccines appear to work on some people, but not others.

Our invention can, we believe, also be used to make worthwhile (or more worthwhile) treatment with existing vaccines in combination with an appropriate dose of antibodies to the antigen of the existing vaccine. Thus a vaccine which a medical practitioner knows exists, but which he also knows is not always very effective, can be made more effective, and more worth trying/using.

We believe that the present invention also gives the vaccinated subject an increased immune response to the antigen A for longer than is conventional.

The speed of response to an antigen of the immune system of a subject may also be increased using the present invention. This may be useful in, for example cases of rabies where it takes days, or weeks (depending where the infected bite is) for the virus to reach the brain of the infected subject. Vaccinating against rabies, after receiving a bite, using the present invention may be more beneficial (and less traumatic for the patient) than simply injecting antigen.

When the present invention is performed on a subject we may prefer to inject the antigen and antibodies in the same injection, or in separate sequential

injections at the same site, or at different sites on the body. We may inject the antigen or antibodies subcutaneously, intravenously, or intramuscularly (or any combination of these).

The injection may be prophylactic or therapeutic.

"Injection" covers any way of introducing the material to the immune system of the subject (e.g. scratching the skin).

The vaccine kit that we propose be used may have the antigen A and the antibody to it in the same container, and the user may draw out a required dose into a syringe. Alternatively we may provide a pre-loaded syringe (preferably one-shot).

Figure 8 shows a kit having a container (e.g. bottle) 10 of antigen A in a pharmacologically acceptable carrier, with a label 12 identifying it, and a container (e.g. bottle) 14 of antibodies to antigen A in a pharmacologically acceptable carrier. This also has a label 16 identifying the contents. The label(s) may give instructions on how to use the kit. The antibodies, antigen, or both may be provided with a suitable adjuvant. The antibodies are preferably present at a dose higher than found in an average human, and may be substantially free of other materials that would elicit an immune response from the patent/subject.

The user takes a predetermined dose of antigen, and a predetermined dose of antibody (possibly in the same syringe) and injects them. Even where they are in different syringes he preferably injects them one straight after the other, perhaps in the same site, but perhaps in different sites (e.g. one in each arm).

Figure 9 shows a syringe 18.

Figure 10 shows an injector 20 that takes a predetermined dose of antigen 10' and antibody 14', the contents of the vials being separate but being mixed before or during injection of their contents.

Figure 11 shows a kit having 10 pairs of vials and is suitable to inject ten patients.

It will be appreciated that the antibodies administered to the source will be at a level such as to have the desired effect. We have indicated the level we found to have a significant effect in adult sheep. It is a matter of limited trial and error to determine a level that works in other sources. Similarly, some degree of experimentation with the level of antigen administered may optimise the response in other sources. Even with sheep, each sheep is genetically different and so there are some variations on the effectiveness of the invention. Different flocks of sheep may respond better to slightly different regimes.

CLAIMS

1. A method of increasing the titre and/or affinity of antibodies in a sample extracted from an antibody source, the method comprising having an antibody producing source and administering to it an antigen, antigen A, and also administering to it antibodies to antigen A (at a suitable dose), thereby causing the source to produce antibodies to antigen A that are of greater affinity than it would do if no antibodies to antigen A were administered to it.

2. The method of Claim 1 in which the antibodies are administered at a dose of 0.001μg to 15 mg.

3. The method of claim 1 or claim 2 in which the source is an animal, preferably a mammal, such as a sheep.

4. The method according to claim 3 in which the animal is a human or a primate.

5. The method according to any preceding claim in which the antibodies are humanised.

6. The method according to any preceding claim in which the antibodies are species specific.

7. The method of any one of claims 1 to 6 in which the administrations of the antigen A and the antibodies to it take place at about the same time, or within a matter of days of each other.

8. A method according to any of claims 1 to 7 in which there is a first administration to the source (simultaneously or within a short time of each

other) of antigen A and/or of antibodies to antigen A, and a second administration of antigen A and/or antibodies to antigen A, the second administration taking place after the first.

9. A method according to claim 8 in which the second administration occurs 1,2,3,4 or 5 days or more after the first is completed.

10. A method according to claim 8 in which the second administration occurs three to ten weeks after the first (or even longer after).

11. A method according to any one of claims 8 to 10 in which the second administration has either (a) antibodies that are of a higher affinity than those administered during the first administration, or (b) a larger dose of antibodies, or (c) both (a) and (b).

12. A method according to any one of claims 8 to 11 in which the second administration has antigen at a larger dose than administered in the first administration.

13. A method according to any one of claims 8 to 12 in which there is a third administration, or subsequent, administrations of antibody and/or antigen doses, after the second administration.

14. A method according to any preceding claim in which the administered antibodies and/or the antigen, are administered intravenously.

15. A method according to any preceding claim in which the source is a cell culture.

16. A method according to any preceding claim in which the source is tolerised to another, or a plurality of other, antigens.

17. A method according to any preceding claim in which the source has administered to it another, or a plurality of other, antibodies to antigens other than the antigen to which the desired antibodies being produced respond.

18. A method according to any preceding claim in which the antibodies administered to the source have a high affinity.

19. A method according to any preceding claim in which the antibodies administered to the source, at least at the first administration, are administered so as to be at a low level in the source of the administration so as not to switch off B-cell production of those antibodies.

20. A method according to any preceding claim in which it is ensured that the source has not previously encountered the selected antigen so as to ensure naive B-cells.

21. A method according to any preceding claim that is a method of increasing the titre of antibodies produced by a source.

22. A method according to any one of claims 1 to 20 that is a method of increasing the affinity of antibodies produced by a source.

23. A method of producing antibodies substantially as described herein.

24. An immunological test kit adapted to diagnose a disorder that is indicated in a sample taken from a patient by the presence of, absence of, or

variation in the amount of an antigen, the kit comprising antibodies to said antigen that have been made in accordance with any one of claims 1 to 23.

25. A kit according to claim 24 which has instructions on how to use it.

26. A kit according to claims 24 or 25 which has control material and/or reference standard material provided in it.

27. A diagnostic test kit substantially as herein described.

28. A preparation for use in a test to identify the presence of an antigen, the preparation having antibodies of high affinity made in accordance with any one of claims 1 to 23.

29. A preparation according to claim 28 which is labelled with its purpose.

30. The use of a preparation including an antibody to an antigen in the production from a source of an extract/sample having high/increased affinity antibodies.

31. The use of a preparation including an antibody to an antigen in the production from a source of an extract/sample having high/increased titre of antibodies.

32. The use of X to do Y, where
x is antibody and antigen, and
y is (a) increased titre
or (b) increase affinity.

33. The use of an antibody (antibody 1) to an antigen (antigen 1) in an administration to an animal or culture, along with antigen 1, in the preparation of a sample extracted from the animal or cell culture, and subsequent use of the sample in the preparation of a diagnostic reagent for use in the identification of the presence of the antigen 1 in a test sample from a test subject.

34. The use of an antibody (antibody 1) to an antigen (antigen 1) in an administration to an animal or cell culture, along with antigen 1, in the preparation of a sample extracted from the animal or cell culture and, the subsequent use of said sample in the preparation of a medicament for use in the treatment of a disease, the disease preferably having an antigen, antigen 1, associated with it.

35. A method of vaccinating or inoculating an animal (e.g. human) against a disease D, the method comprising vaccinating with a vaccine against disease D having antigen of disease D, or closely associated with an antigen of disease D, and also introducing to the animal antibodies to the antigen vaccinated.

36. A method according to claim 35 in which the antibodies are introduced with a dosage of 0.001μg to 15 mg.

37. A method according to claim 35 or claim 36 in which the antibodies administered have an affinity of at least 10^{-9} L/mol, and most preferably of 10^{-10} L/mol or above.

38. A method of vaccinating or inoculating substantially as described herein.

39. A method of improving the performance of a vaccination or inoculation, the method comprising giving a vaccination of vaccine material that hitherto did not work as well as it might have and also administering antibodies to at

least some of the vaccine material, thereby generating higher affinity antibodies in the vaccinated subject.

40. A vaccine (or other medicament containing an antigen) comprising a component that has antigen, antigen A, of at least a part of the structure of a bacteria, virus or other harmful agent, and a component that comprises antibodies to antigen A.

41. A vaccine according to claim 40 in which the amount of the antibodies is 0.001μg to 15 mg.

42. A vaccine according to claim 40 or claim 41 in which the antigen A and the antibodies to it are in separate pharmacologically acceptable carriers, which may be adapted to mix during the injection process.

43. A vaccine according to any one of claims 40 to 42 in which the antigen A and the antibodies to it are present in the same pharmacologically acceptable carrier.

44. A vaccine according to any one of claims 40 to 43 which is labelled as having an antibody in it.

45. A vaccine according to any one of claims 40 to 44 which is provided in a predetermined dose in a cartridge or vial for a syringe or injector, or is pre-provided in a syringe or injector.

46. A vaccine according to any one of claims 40 to 45 in which the antibodies have an affinity of 10^{-9} L/mol or above.

47. A vaccine according to any one of claims 40 to 46 in which the antibodies have an affinity of 10^{-10} L/mol or above.

48. A vaccine according to any one of claims 40 to 47 in which the antibodies have an affinity of 10^{-11} L/mol or above.

49. A vaccine according to any one of claims 40 to 48 which is adapted to treat disease X, and the antigen A is at least a part, and perhaps substantially all, of the protein structure of the agents that cause disease X;
where X is from the list:-

Rubella
Rabies
Diphtheria
Tetanus
Whooping Cough
Typhoid
Hepatitis A
Hepatitis B
Hepatitis C
Tuberculosis
Chicken Pox
Mumps
Scarlet Fever
Yellow Fever
Aids
CJD
Polio
Cholera

50. A vaccine according to any one of claims 40 to 49 which also includes an adjuvant.

51. A vaccine substantially as described herein.

52. A kit comprising a vaccine in accordance with any one of claims 40 to 51 and one or more of: an injector; or instructions on how to use the vaccine; or a plurality of containers containing a predetermined amount of vaccine adapted to treat a plurality of patients.

53. The use of both antigen A and antibodies to antigen A in the preparation of a medicament or vaccine for the treatment (prophylactic or therapeutic) of disease X, where the agent that causes disease X has antigen A (or an antigen very similar) in its protein structure.

54. The use of both antigen A and antibodies to antigen A according to claim 53 where disease X is not a disease in its normal sense but is instead a disorder associated with the present of antigen A (e.g. where antigen A is a toxin for example a snake venom, or the results of a drug overdose).

55. In a series of planned injections at spaced apart times intended to vaccinate against a disease X, that has associated with it an agent having a structure of antigen A, the administration to a subject being vaccinated of both the antigen A and antibodies to the antigen A in order to improve the immune response of the subject to the first injection (or early injections in the planned series) thereby avoiding the need for at least one of the later traditionally performed injections.

56. In a series of planned injections according to claim 55, the use of the administration of both antigen A and antibodies to antigen A to reduce the number of injections required from 3 to 2.

57. In a series of planned injections according to claim 55, the use of the administration of both antigen A and antibodies to antigen A to:-

a) reduce the number of planned injections to 1; or

b) reduce the number of planned injections by 1 (or more) (for example from 4 to 3, 3 to 2, or 2 to 1)

58. The use of an antibody (antibody 1) to an antigen (antigen 1) in an administration to an animal or cell culture, along with antigen 1, in the preparation of a sample extracted from the animal or cell culture and, the subsequent use of said sample in the preparation of a medicament for use in the treatment of a disease, the disease preferably having an antigen, antigen 1, associated with it.

59. A method of improving an inoculate comprising ensuring that the inoculate has both an antigen and antibodies to the antigen.

60. A method of diagnosing localised disorders (e.g. tumours) comprising introducing into the patient antibodies that are specific to one or more epitopes of the disorder (e.g. tumour) and having the antibodies associated with a readily detectable agent so that when a diagnostic scan is performed the readily detectable agent is localised at the site of the disorder to make it detectable, and wherein the efficiency of the method is increased by using antibodies that are of high affinity.

61. A method according to claim 60 in which the antibodies are of a higher affinity than can be obtained simply by introducing into a naive antibody source antigen (of the desired epitopes) and extracting the antibodies so generated.

62. A method according to claim 60 or claim 61 in which the antibodies have an affinity that is 10^{-9} L/mol or above.

63. The use of high affinity antibodies in the preparation of a diagnostic substance for the diagnosis of (or improved imaging of) a localised disorder (e.g. tumour), the antibodies having been made in accordance with any of claims 1 to 23.

64. A method of targeting the delivery of a therapeutic substance to some cells but not other cells in vivo, the method comprising using antibodies specific to one or more epitopes of said some cells, (which epitopes are not present on said other cells), and associating with the antibodies the therapeutic substance, the method further comprising using high affinity antibodies made in accordance with any one of claims 1 to 23.

65. A method according to claim 64, in which the antibodies are of higher affinity than can usefully be obtained by administering the epitopes to a naive antibody source and extracting the antibodies to the epitopes from the source.

66. A method according to claim 64 or claim 65 in which the antibodies have an affinity of at least 10^{-9} L/mol.

67. The use of high affinity antibodies in the preparation of a medicament for the treatment of a disease or disorder, the high affinity antibodies concentrating the medicament in specific regions better than with conventional

lower affinity antibodies, and the antibodies being made in accordance with any one of claims 1 to 23.

68. A method of diagnosing a disorder in which a protein is produced, or is absent or diminished, comprising taking a test sample from a subject animal and using an antibody to the protein to identify its presence, and in which the antibody has been produced in accordance with any of claims 1 to 23.

69. A product produced by the method of any preceding claim.

70. A medicament or therapeutic substance for use in the treatment of disorder associated with an antigen, antigen A, the medicament having antibodies to antigen A, said antibodies being of high affinity and being produced in accordance with any one of claims 1 to 69 and being present at a level such as to have a beneficial result when the medicament is administered to a patient.

71. A medicament or therapeutic substance according to claim 70 in which there is also a physiologically active substance.

72. A medicament or therapeutic substance according to claim 71 in which the physiologically active substance is associated with the antibodies (e.g. conjugated with them).

73. A medicament according to any one of claims 70 to 72 which is substantially free of other physiologically active substances (beyond said antibodies and if present said [selected] physiologically active substance).

SHP. NO.	GROUP	TRMT.	BT	BB	HT	HB	RM5	RM1	RM5x5	Sheep IgG
C903	A	25	1871	32	31655	1187	8.7	5.9	75.7	30.4
D001	A	25	1300	60	13970	937	6.3	4.1	39.7	30.4
D011	A	25	1448	35	24402	907	7.2	4.8	51.8	32.2
D021	A	25	1505	40	27959	926	7.8	5.4	60.8	29.6
D025	A	25	1426	15	28293	890	7.5	5	56.3	27.8
D039	B	1	1601	58	28280	1087	8.6	5.8	74	33.2
D040	B	1	1372	46	27069	1024	7.5	5	56.3	26
D044	B	1	1412	61	26621	1067	7.3	4.8	53.3	35
D058	B	1	1586	71	26882	958	8	5.1	64	22.8
D067	B	1	1217	58	12593	932	6.5	4.2	42.3	24.4
D061	C	0.1	1811	66	31965	1085	9	5.5	81	28.6
D064	C	0.1	1555	36	28109	967	7.7	5.1	59.3	32.2
D072	C	0.1	1564	50	26578	917	8	5.4	64	27.8
D076	C	0.1	1474	39	25303	984	7.7	5.1	59.3	26
D078	C	0.1	1535	43	27493	901	7.9	5.5	62.4	32.2
D080	D	0.01	1612	38	27318	994	7.5	5.2	56.3	27.8
D081	D	0.01	1415	42	20003	1011	6.7	4.5	44.9	24.4
D082	D	0.01	1496	25	23166	2973	7.2	4.8	51.8	24.4
D084	D	0.01	1662	60	27537	1143	7.5	5.2	56.3	28.6
D085	D	0.01	1642	22	12039	788	5.5	4	30.3	27
D089	E	0.001	1787	10	28502	849	8.1	5.2	65.6	31.4
D091	E	0.001	1233	10	10920	709	6	4.2	36	26.0
D092	E	0.001	1550	100	27613	1389	7.5	4.9	56.3	29.2
Y450	E	0.001	1265	24	11095	852	5.7	4	32.5	33.2
Y451	E	0.001	1378	26	24987	878	7.5	4.7	56.3	29.6
E662	F	0	1473	33	27377	896	7.8	5.2	60.8	30.4
E663	F	0	1513	27	25786	796	7.6	5	57.8	28.6
E664	F	0	1514	50	28843	1298	7.8	5	60.8	28.6
E665	F	0	1410	24	8340	693	5.5	3.7	30.7	25.2
E666	F	0	1503	31	23465	916	7.3	4.5	53.3	42.3

Fig 1

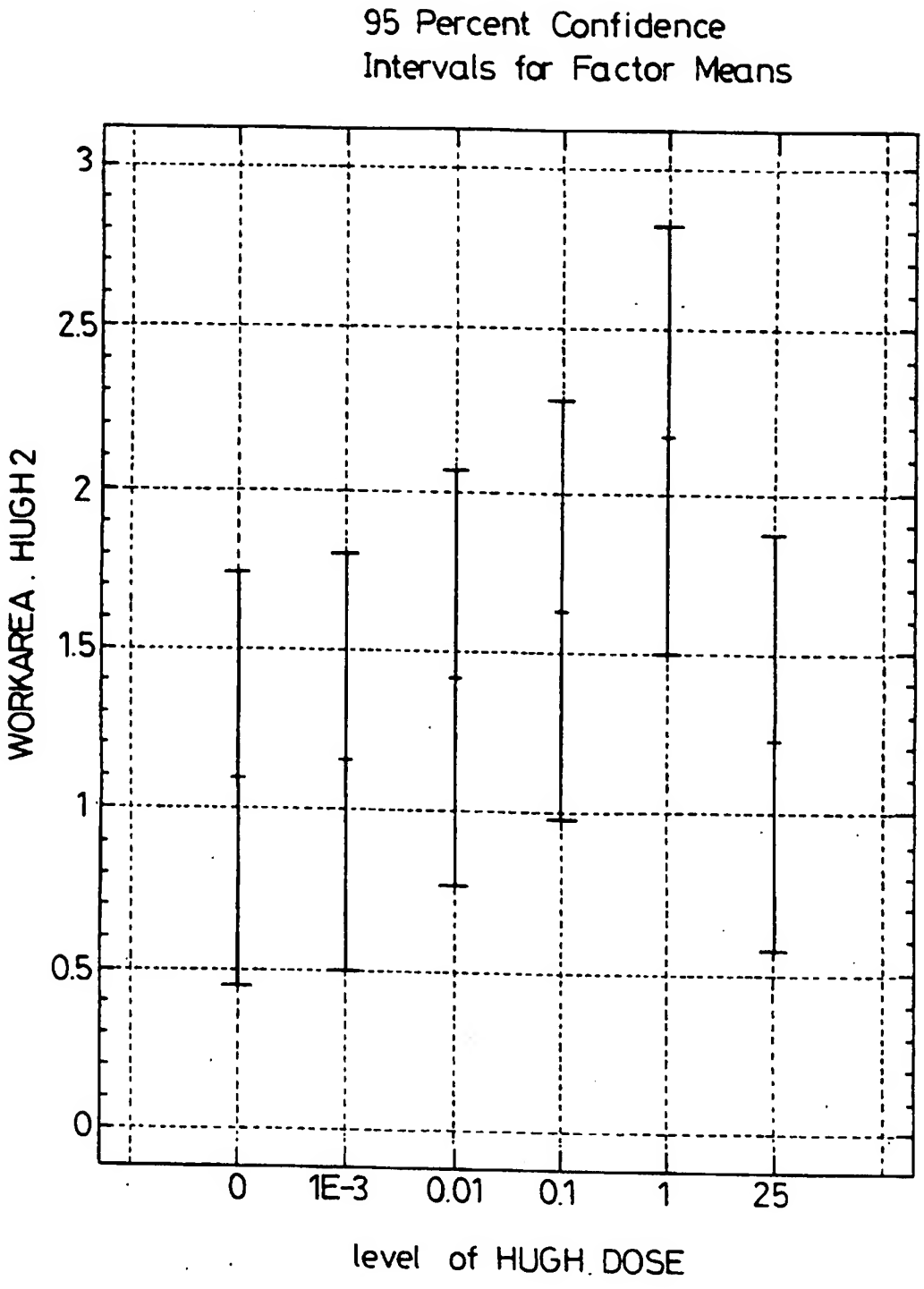

95 Percent Confidence
Intervals for Factor Means
WORKAREA. HUGH 2
3
2.5
2
1.5
1
0.5
0
0
1E-3
0.01
0.1
1
25
level of HUGH. DOSE

Fig 2

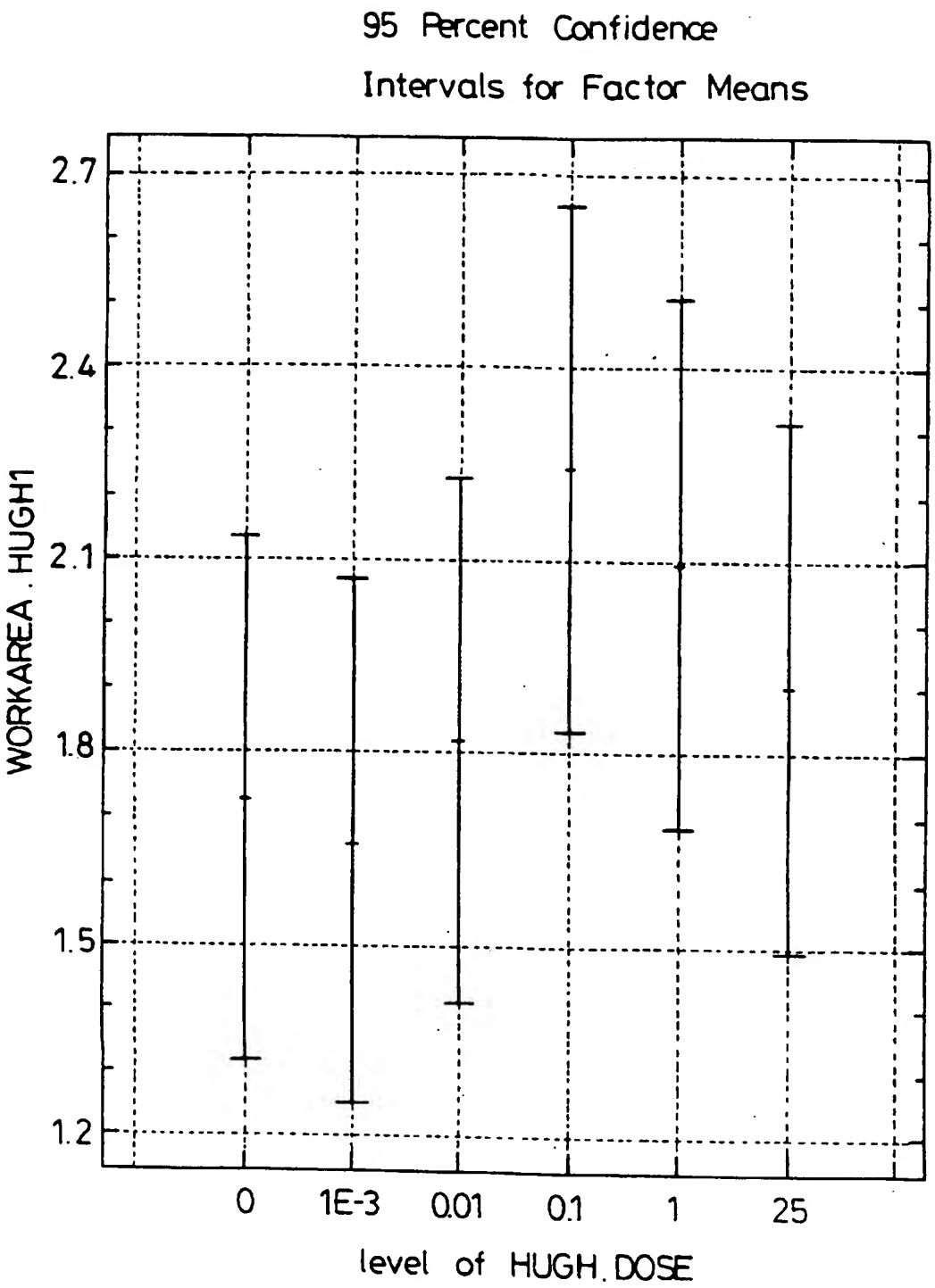

95 Percent Confidence
Intervals for Factor Means
WORKAREA.HUGH1
2.7
2.4
2.1
1.8
1.5
1.2
0
1E-3
0.01
0.1
1
25
level of HUGH.DOSE

Fig 3

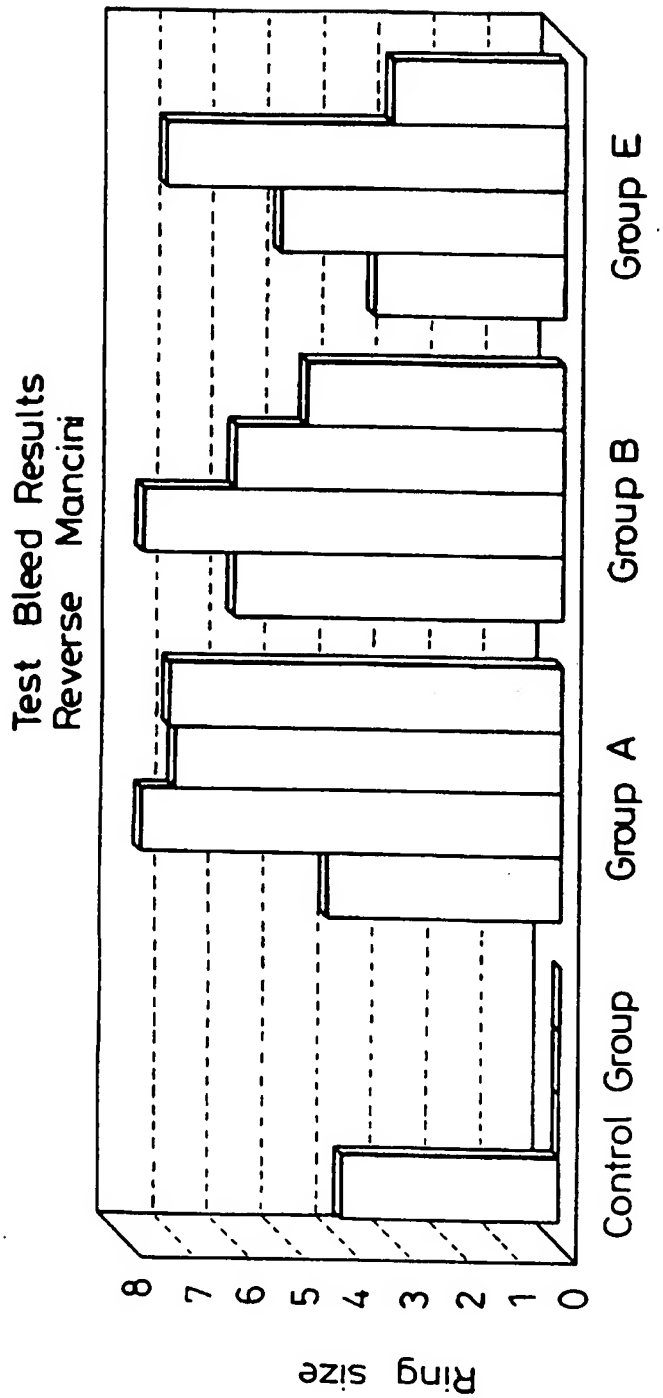
PROTOCOL : 28 virgin sheep immunised as follows:

Control Group	Sheep	G419, G420, G421, G422
	Prime	Day 0 100μg IgGF(c) AG 2399
	Boost	Day 42 20μg IgGF(c) AG 2399
Group A	Sheep	G403, G404, G405, G406
	Prime	Day 0 100μg IgGF(c) AG 2399
	I.V. ab	Day 8 25μg a-IgGF(c) (IgG a/s) AB 104
	Boost	Day 42 20μg IgGF(c) AG 2399
Group B	Sheep	G407, G408, G409, G410
	Prime	Day 0 500μg IgGF(c) AG 2399
	I.V. ab	Day 8 125μg a-IgGF(c) (IgG a/s) AB 104
	Boost	Day 42 100μg IgGF(c) AG 2399
Group E	Sheep	G399, G400, G401, G402
	Prime	Day 0 500μg IgGF(c) AG 2399
	I.V. ab	Day 8 15μg a-IgGF(c) (IgG a/s) AB 104
	Boost	Day 21 100μg IgGF(c) AG 2399

Fig 4

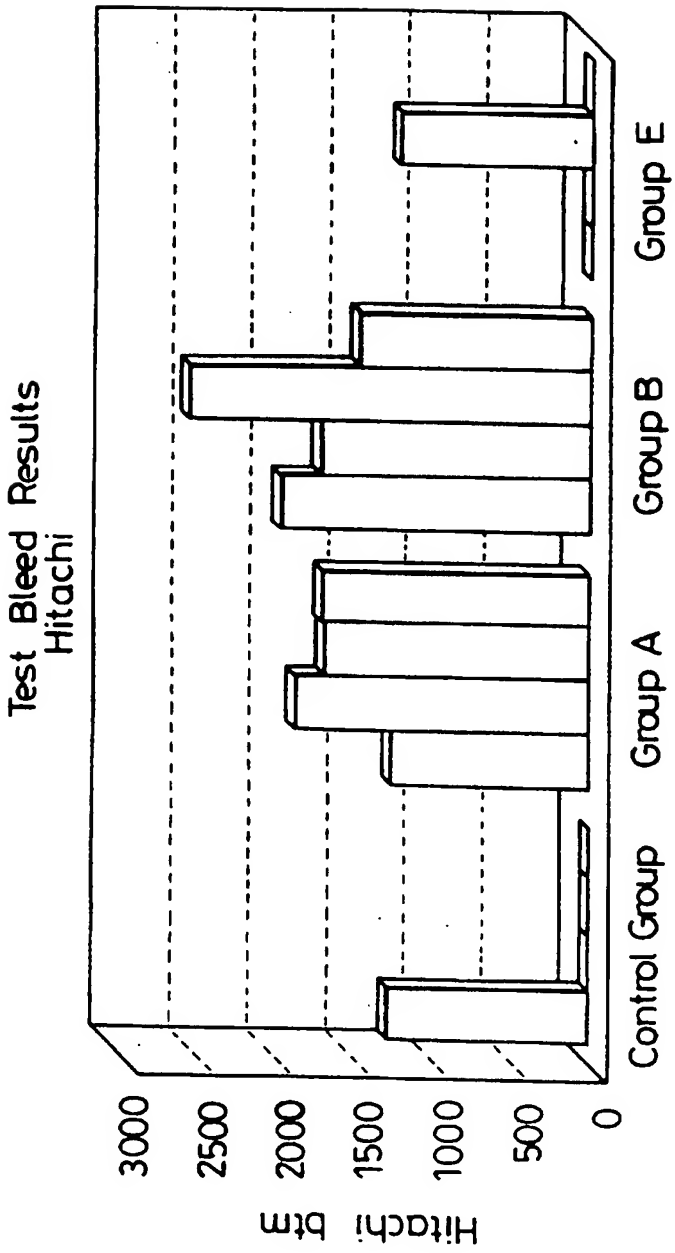
Sheep	Group	RM		IEP	Hitachi Results			
		1µl	5µl		sal. blank	mira btm	mira top	top OD
	Dako	4.0	6.5					
G419	control	-	4.0	specific	765	1292	1305	2631
G420		-	-	specific	not tested			
G421		-	-	specific	not tested			
G422		-	-	specific	not tested			
G403	A	3.3	4.3	contam.	609	1265	2144	3126
G404		5.6	7.7	specific	942	1877	28750	31454
G405		5.1	7.1	specific	718	1690	26509	28336
G406		4.7	7.2	specific	725	1703	24760	26144
G407	B	4.0	6.0	specific	857	1970	14294	14294
G408		5.7	7.7	specific	662	1716	30783	32329
G409		4.4	6.0	specific	1567	2554	18618	19261
G410		3.5	4.7	specific	906	1472	3982	5120
G399	E	-	3.5	specific	not tested			
G400		3.7	5.2	specific	not tested			
G401		5.2	7.3	specific	501	1218	21031	21170
G402		-	3.2	specific	not tested			

Fig 5



	Sheep 1	Sheep 2	Sheep 3	Sheep 4
Control Group	4			
Group A	4.3	7.7	7.1	7.2
Group B	6	7.7	6	4.7
Group E	3.5	5.2	7.3	3.2

Fig 6



	Sheep 1	Sheep 2	Sheep 3	Sheep 4
Control Group	1292			
Group A	1265	1877	1690	1703
Group B	1970	1716	2554	1472
Group E			1218	

Fig 7

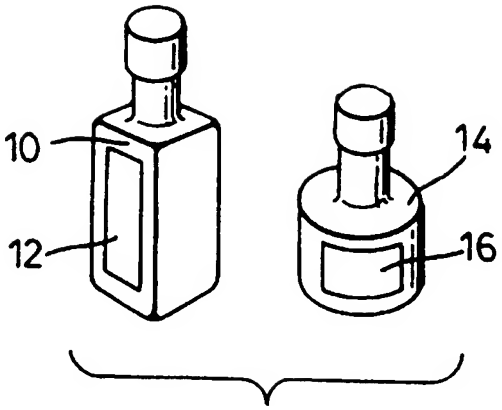


Fig 8

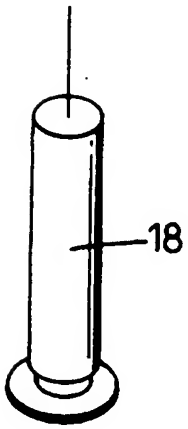


Fig 9

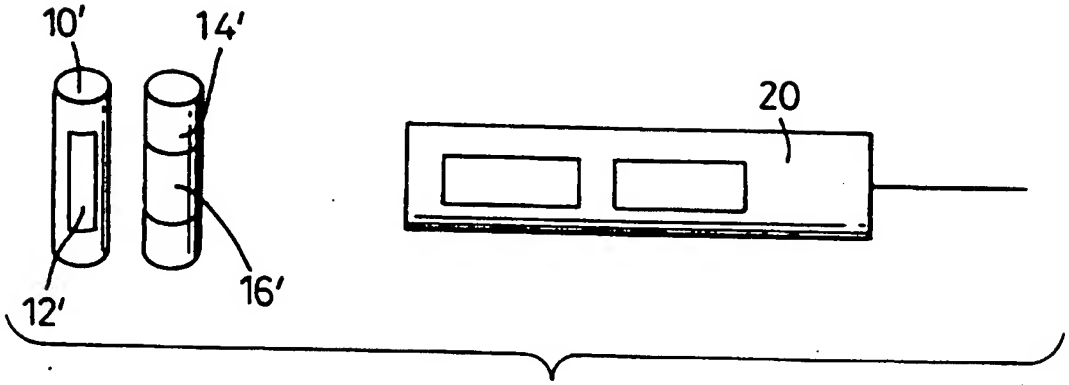


Fig 10

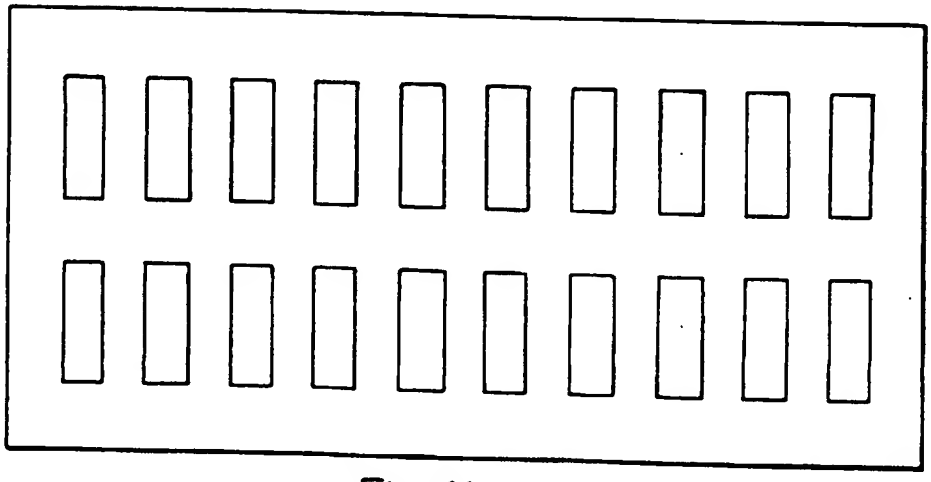


Fig 11